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Southernmost record of *Liparis montagui* (Donovan, 1804) (Perciformes, Liparidae) in European waters (central Portugal), with genetic validation

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Abstract. In January 2022, the presence of adult *Liparis montagui* (Donovan, 1804) was documented at its southernmost point along the west coast of Portugal. The species was identified through both morphological and genetic barcoding analyses. This observation, part of an ongoing long-term coastal survey, suggests that *L. montagui* may be a transient species, influenced by complex climate and oceanic interactions along the western Iberian coast. This finding stresses the importance of long-term ecological studies and regular field surveys in understanding species distribution and the effects of climate change on marine biodiversity.

Key words. Central Portugal, cold-water species, distribution range, first occurrence, Montagu's Seasnail

Duarte-Coelho P, Miranda C, Pereira A, Vasco-Rodrigues N, Almada F (2025) Southernmost record of *Liparis montagui* (Donovan, 1804) (Perciformes, Liparidae) in European waters (central Portugal), with genetic validation. Check List 21 (2): 400–405. https://doi.org/10.15560/21.2.400

INTRODUCTION

Liparis montagui (Donovan, 1804), Montagu's Seasnail, is a small clinging fish usually found in intertidal (Aguirrezabalaga et al. 1988) and subtidal areas to a depth of 30 m (Gibson 1972; Hauksson 2000; Froese and Pauly 2022). It inhabits small holes and crevices, especially in kelp forests (Gordon 1983; Norderhaug et al. 2005) and prefers exposed shores rather than sheltered waters (Gibson 1972). The maximum size of this species varies according to different bibliographic sources, ranging from 10 cm (Debelius 1997) or 12 cm (Derweduwen et al. 2014) to 15.5 cm (Wilhelms 2013). Notably, the maximum size tends to increase with latitude and is larger towards the north (Lin and Costello 2023). According to Dunne (1981), this species achieves maturity at approximately 3 cm and the average size along the British coast ranges from 4 to 6 cm. No other information on the reproduction of this species was found, although other liparids of a similar maximum size also become sexually mature at 3 cm (Able and Musick 1976; Stein 1980). Their diet consists mainly of amphipods (Gordon 1983) and small decapods (Derweduwen et al. 2014).

The distribution of this species encompasses the North Sea, around the British Isles, the Norwegian Sea, southwestern Barents Sea, and around southern Iceland (Ahyong et al. 2022; Froese and Pauly 2022), showing a marked preference for cold waters (Matallanas 1983) (Figure 1). Nevertheless, there are records of larvae from further south, in Galicia (Spain) and northern Portugal near the Spanish border (Matallanas 1983; Ré et al. 1985; Bañon et al. 2010). Along this southernmost limit of the geographic range of *L. montagui*, larvae were found at very low abundances (Ré et al. 1985; Ré 1999; Ramos et al. 2006; Pereira 2021). On one occasion, five juveniles were reported in northern Portugal (Ré et al. 1985), although no physical samples were preserved, and no genetic analysis was performed.

During an ongoing long-term rocky coastal survey along the western Portuguese coast, two individuals of an undetermined fish species were collected in Cabo Raso, Cascais, Portugal. This study aims to identify these individuals using morphological and genetic barcoding.

OPEN ACCESS

Academic editor: Paramasivam Kodeeswaran
Received: 13 January 2025
Accepted: 24 March 2025
Published: 1 April 2025

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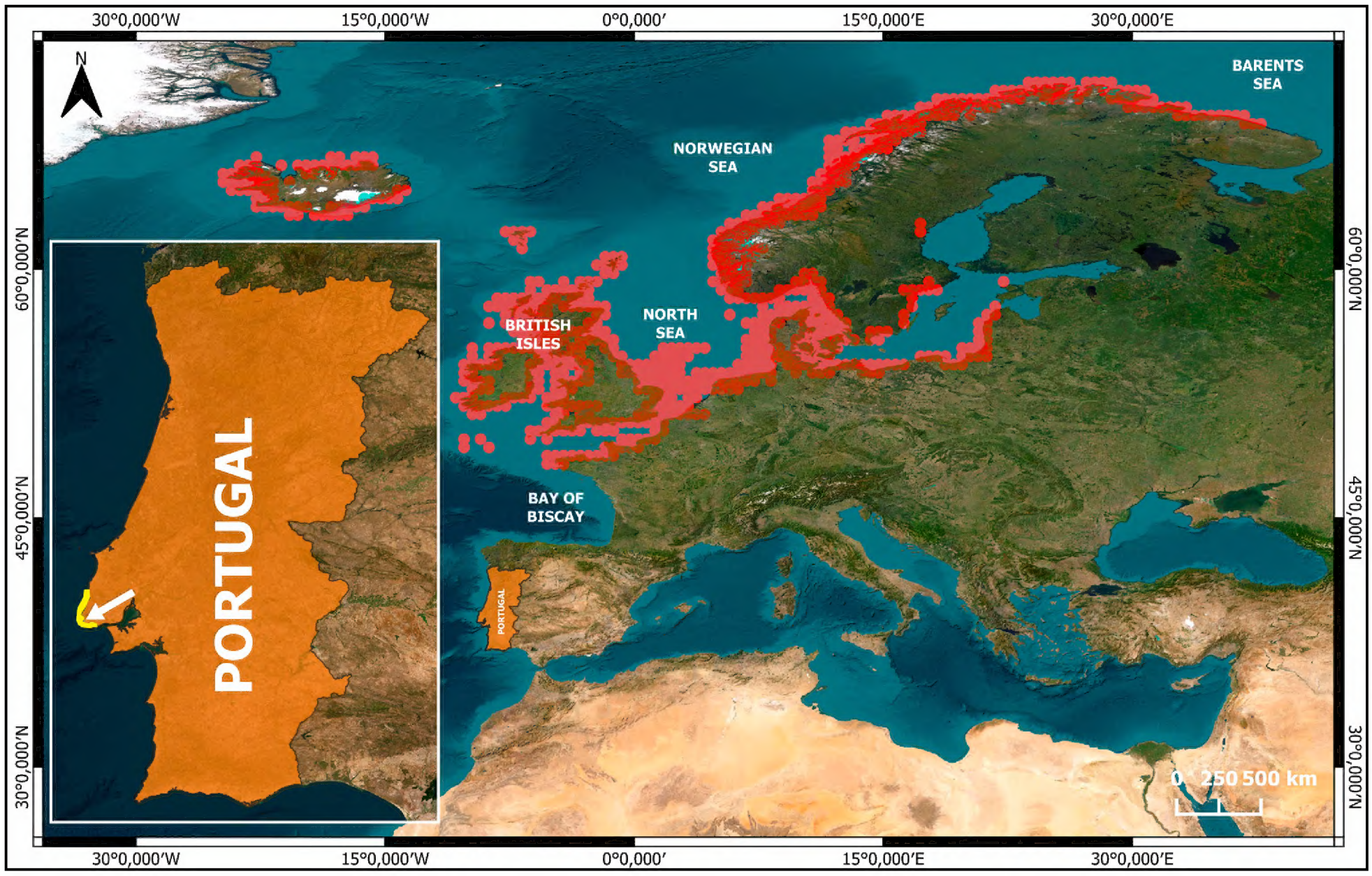

Figure 1. Map showcasing the native range of ***Liparis montagui*** is illustrated in red, based on data points from FishBase. The newly identified country is marked in orange, with the sampling site indicated by a white arrow (Cabo Raso, Cascais, Portugal). A 50 km area monitored through long-term observation is highlighted in yellow.

METHODS

This study originates from a long-term rocky coastal survey that has been ongoing since 2019, covering over 50 km of the western Portuguese coast. The sampling site for this report is at Cabo Raso, Cascais, Portugal (34°42'32.77'N, 009°29'11.28"W) (Figure 1). The sampling surveys for vertebrates and invertebrates involved fixed 50 m^2 transects, which were conducted monthly at each sampling site. On 3 January 2022, two fish samples were collected, photographed *in vivo*, and a small portion of fin tissue from the larger individual was collected and preserved in 96% ethanol. The smaller individual was collected (authorized by the National Maritime Authority under permit 445/2022), transported to our fish facility, used for morphological confirmation (using a ZEISS Stemi 508 stereomicroscope), anesthetized, and preserved in 96% ethanol for reference keeping. Voucher specimens are stored at MARE-ISPA.

The DNA extraction and amplification were performed using the Sigma-Aldrich/Merck REDExtract-N-Amp Tissue PCR commercial kit using a thermal cycle (analytikjena, Biometra TOne). Two mitochondrial genes were analyzed: the 16S ribosomal subunit and Cytochrome Oxidase I (COI). The primers used for COI were COI-F1 FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and COI-R2 FishR2 (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3') (Ward et al. 2005). For 16S rDNA, the primers were 16SFor (5'-AAGCCTCGCCTGTTTACCAA-3') and 16SRev (5'-CTGAACTCAGATCACGTAGG-3') (Almada et al. 2005). The amplification protocols are described in Ward et al. (2005) for COI and Almada et al. (2005) for 16S. Sequences were edited, assembled and aligned using CodonCode Aligner V.11.0.2 and a BLAST search performed in NCBI for identification confirmation.

RESULTS

Liparis montagui (Donovan, 1804)

New records. PORTUGAL — **Cascais** • Cabo Raso; 34°42'32.77"N, 009°29'11.28"W; intertidal transect survey; 02.I.2022; Duarte-Coelho and Almada leg. GenBank: PQ640289 and PQ640290 (COI), PQ661180 and PQ661181 (16S); 2 specimens preserved in ethanol, sex indet., 03012022P5M1T001 and 03012022P5M1T005.

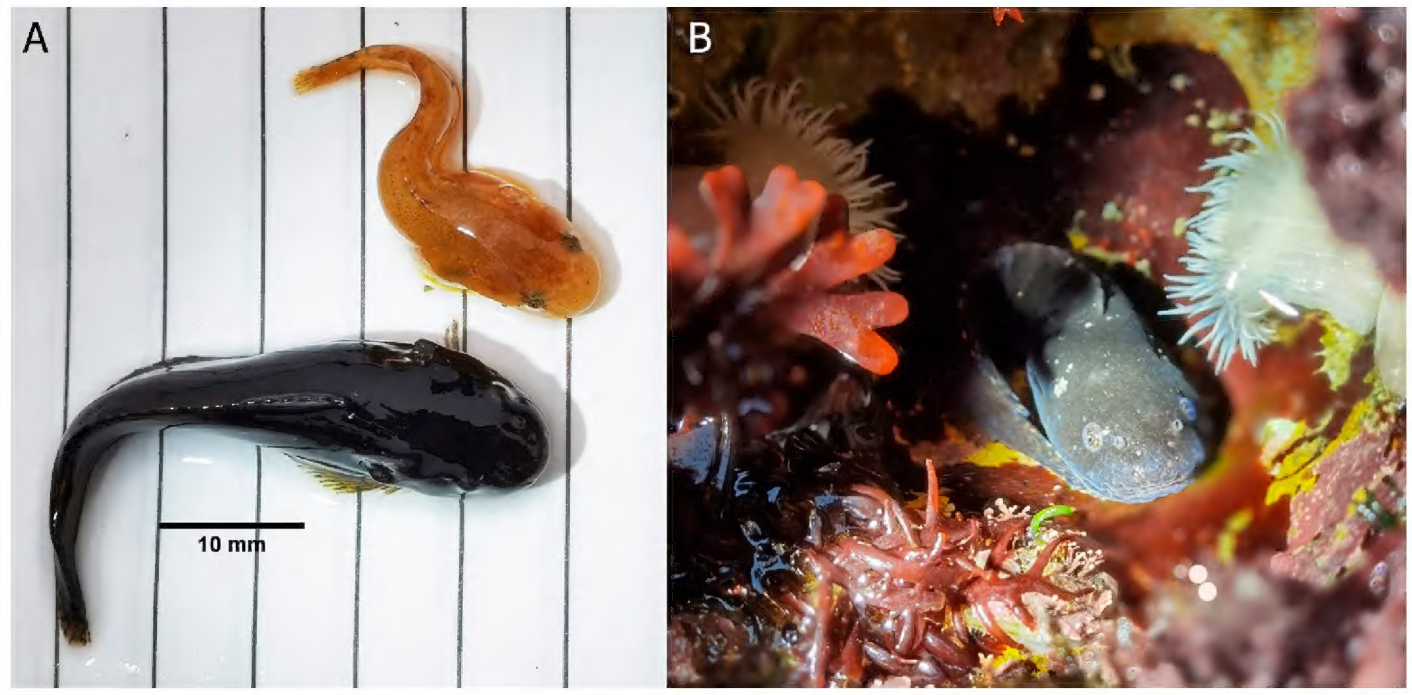

Figure 2. *Liparis montagui*. **A.** Specimens displaying two distinct color variations. **B.** The larger (dark) specimen in its natural microenvironment.

Identification. The fish were first identified using their morphological characteristics and later confirmed through DNA barcoding. Both specimens belong to the family Liparidae (Jenkins 1942; Wheeler 1978; Lloris 2015). The genus *Liparis* was determined by the following traits: a small, round-bodied fish with a ventral sucker, two pairs of nostrils, and the presence of pseudobranchia (Wheeler 1978; Lloris 2015). According to Lloris (2015), the absence of sinuous lines along the body is a diagnostic feature that distinguishes *L. montagui* from *L. liparis*.

One specimen was dark grey (Figure 2A, B) with a total length of 4.65 cm. GenBank accession numbers PQ640289 (COI) and PQ661180 (16S). Only a small portion of tissue was collected (ref. 03012022P5M1T001), and the individual was released. The second specimen was yellow/orange (Figure 2A), with a total length of 3.05 cm. GenBank accession numbers PQ640289 (COI) and PQ661180 (16S). Specimen was preserved for reference keeping and stored at MARE-ISPA (ref. 03012022P5M1T005).

A partial sequence of 652 base pairs (bp) of the COI gene (GenBank accession numbers PQ640289 and PQ640290) and a partial sequence of 561 bp of the 16S rRNA gene (GenBank accession numbers PQ661180 and PQ661181) were obtained for each individual. Both individuals shared the same COI haplotype but had two different 16S haplotypes, separated by two mutations. Identification using both markers confirmed that both specimens belong to *Liparis montagui*. For the 16S gene, haplotype 1 showed a 100% percentage identity with 99% query cover, while haplotype 2 showed a 99.64% percentage identity with 99% query cover. For the COI gene, both samples exhibited a 99.53% percentage identity with a 98% query cover.

DISCUSSION

Following our observations, Costa et al. (2022) reported another individual of the same species in April 2022 on the western Iberian coast, approximately 300 km north of our findings. These sightings were flanked by extensive periods with no observations of adult *Liparis montagui* along the West Iberian coast. This species appears to be rare even within its central distribution range (Dunne 1981; Gordon 1983; Rogers and Millner 1996; Derweduwen et al. 2014; Iglésias et al. 2021) when compared to its sister species, *Liparis liparis* (Halliday 1969; Greenwood et al. 2002). Its abundance fluctuates significantly over time (Dunne 1981), and some studies suggest a decrease in abundance along its distribution range (Genner et al. 2010; Gibson et al. 1993). For instance, the first record of *L. montagui* in Belgian waters was only reported in 2014 (Derweduwen et al. 2014) and records are very scarce in France (Iglésias et al. 2019). Additionally, fish of the genus *Liparis* are challenging to identify using morphological characteristics and are poorly studied taxonomically (Vogt and Chernova 2015). The two individuals captured exhibited distinct color morphotypes. This species is known to display a wide range of patterns and colors, including dark grey, brown, olivaceous, orange, and yellow (Garman 1892). These variations are likely influenced by environmental factors, serving as a mechanism for camouflage and blending into their surroundings. The proximity of the two individuals and their capture during the breeding season (Dunne 1981; Quero et al. 1980) suggests, although no sexing was performed, that they might have been breeding. While the maturity of the smaller individual may be uncertain, the larger one, measuring nearly 5 cm, is undoubtedly an adult. Although data on the maturity size of this species remain scarce, Dune (1981) previously noted that they typically reach maturity at a size of 3 cm, as mentioned in the introduction. We suggest that individuals at the southern edge of their distribution experience increased environmental stress, leading to smaller body sizes and a faster reproductive strategy (Thorsen 2010). Moreover, research from multiple authors highlights a gradual reduction in the maximum length of this species toward the south, providing further support for this hypothesis (Lin and Costello 2023).

The scarcity of records for this species along the Portuguese coast may be explained by two alternative hypotheses: this species is present, although unreported, in the entire region, or is transient in the southernmost region of its geographical range.

The first hypothesis is that this species is present but remains largely unreported along the western Iberian coast (approximately 700 km long). The fact that this is a small cryptic species (Hanchet et al. 2013), with mainly nocturnal habits (Gordon 1983), may justify the lack of records of adult stages along the Portuguese coast. However, arguing against this hypothesis, there has been abundant research focused on small cryptic fish species from inshore habitats along west Iberia. Some of these species share identical morphological traits, cryptic behavior, and habitat preferences with *L. montagui* and were followed by authors for more than a decade (e.g. Almada et al. 2008; Martins et al. 2017; Francisco et al. 2021). Furthermore, an ongoing long-term inshore survey has only been able to identify the two individuals reported in this study.

A second hypothesis is that, although the presence of this species along the western Iberian coast cannot be discarded, the detail and the geographic scope of these studies together with this ongoing survey, suggest that these fish are either absent or very rare in this region. Their report on specific periods and the lack of success finding more individuals since then suggest that inshore marine communities are still poorly known regarding their ecology and dynamics. This is especially true in complex biogeographic areas, such as the Iberian coast, which transition between cold- and warm-water communities (Almada et al. 2013). Species that are transient or rare provide a striking example of the importance of coupling long-term ecological studies with a regional plan to monitor environmental factors and to address changes in local biodiversity. This may help us follow habitat expansions or contractions of different species in a context of fast climate change.

The presence of this cold-water species at this southernmost location, in contrast with a steady tropicalization of temperate fish communities (Horta e Costa et al. 2014), highlights our inability to predict future events and reveals the current instability of coastal communities. It also signals the need for further research and long-term ecological surveys to disentangle regional isolated events from global ecological trends (but see Biguino et al. 2023). We propose that a multi-methodology approach, combining long-term monitoring through traditional field sampling techniques with modern advancements such as eDNA studies, would provide a significantly more effective means of assessing the status of our coastal waters.

ACKNOWLEDGEMENTS

We thank Andreia Guilherme, Tassiana Malzone and Francisca Rodrigues for all their support during fieldwork. We would like to extend our gratitude to the reviewers and the academic editor for their valuable insights and dedicated efforts, which have been instrumental in the publication of this manuscript.

ADDITIONAL INFORMATION

Conflict of interest

The authors declare that no competing interests exist.

Ethical statement

The fieldwork and specimen collection complied with the animal welfare laws, guidelines, and policies set by ORBEA (ISPA – Instituto Universitário). The collection was authorized by the National Maritime Authority under approval [445/2022]. The species studied are classified as Least Concern (LC) on the IUCN Red List.

Funding

This study had the support of national funds through Fundação para a Ciência e Tecnologia (FCT), granted to MARE (Marine and Environmental Sciences Center) under the references UIDB/04292 and UIDP/04292.

Coastal biodiversity surveys have also been funded by Activo Bank through an ongoing protocol with the non-profit association MARDIVE (https://www.mardive.org).

F.A. (Frederico Almada) had the support through researcher contract DL57/2016/CP1339/CT0003.

Author contributions

Conceptualization: FA, PDC. Data curation: PDC, CM. Formal analysis: PDC, FA, CM. Funding acquisition: FA. Investigation: PDC, FA, CM. Methodology: PDC, CM, FA. Resources: PDC, CM, FA. Supervision: FA, AP. Validation: FA, NVR, CM, PDC. Writing – original draft: PDC. Writing – review and editing: PDC, FA, AP, NVR, CM.

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Data availability

All data that support the findings of this study are available in the main text.

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